

AMENDMENTS TO THE CLAIMS

1. (currently amended) A method for detecting any one of multiple chromosomal disorders in a single assay, which method comprises the steps of:
 - a. making a polymerase chain reaction (PCR) mixture by mixing in a vessel components comprising:
 - (i) eukaryotic genomic DNA;
 - (ii) a plurality of different pairs of forward and reverse DNA primer oligonucleotides wherein one primer of each different said pair is complementary to a 3' sequence of a targeted segment of a first DNA strand of the eukaryotic DNA and the other primer is complementary to the 3' sequence of the second strand of the targeted segment, the length of the segment of eukaryotic DNA being between about 50 and about 300 base pairs, wherein one of the primers of each different pair has a detectable label attached to its 5' end, wherein of said plurality of different pairs of primers there are pairs targeted to segments of selected different chromosomes of interest, each of which is indicative of a potential chromosomal disorder, and wherein one pair is targeted for a segment of a single control gene which is present on a chromosome other than one on which there is a targeted segment and does not target any chromosome segment that might be indicative of a potential aneuploidy; and
 - (iii) PCR buffers and enzymes necessary to carry out PCR amplification;
 - b. conducting a PCR for between about 5 and about 60 temperature cycles to create amplified PCR products;
 - c. purifying said products of step (b) and obtaining single-stranded DNA having the detectable labels,
 - d. contacting a microarray with products of step (c), which microarray has a plurality of spots that each contain DNA oligonucleotide probes having nucleotide sequences complementary to a nucleotide sequence of one of said strands of each of said targeted segments;

- e. hybridizing said DNA oligonucleotide probes in said microarray and said PCR-amplified label-containing single-stranded products;
- f. detecting the presence and relative quantity of the PCR-amplified products hybridized to the microarray by imaging the microarray; and
- g. diagnosing whether or not a chromosomal disorder exists with respect to one or more of said selected different chromosomes by comparing said imaging of the relevant spots on said microarray for each said targeted segment of a selected chromosome to the imaging of spots relevant to said single control gene ~~and to determine a ratio of such intensities for each of a plurality of said targeted segments, termed an I-ratio, then to results dividing each said I-ratio by an N-ratio~~ obtained from similar testing of multiple samples of genomic DNA of the same gender known to be normal to obtain a C-factor for each, averaging all said C-factors to obtain an average C-factor, then adjusting each said I-ratio by said average C-factor, and then comparing each said adjusted I-ratio to the respective N-ratio to determine if a disorder exists.

2. (canceled)

3. (currently amended) The method according to ~~claim 1~~ claim 22 wherein at least two of the targeted segments of eukaryotic genomic DNA that are selected are associated with potential microdeletions of chromosomal DNA that would give rise to chromosomal disorders selected from the group consisting of:

Williams-Beuren syndrome,
Cri du chat syndrome, and
DiGeorge syndrome.

4. (currently amended) The method according to ~~claim 1~~ claim 22 wherein at least two of the targeted segments are selected to detect chromosomal aberrations selected from the group consisting of trisomy 13, trisomy 18, trisomy 21 and X- and Y-chromosome anomaly.

5. (currently amended) The method according to ~~claim 1~~ claim 22 wherein said detectable labels are color-detectable labels.
6. (original) The method according to claim 5 wherein said color-detectable labels are attached to the reverse primers and the forward primer of each pair has phosphate at its 5' end.
7. (original) The method of claim 6 wherein said detectable labels are fluorescent dyes.
8. (original) The method of claim 1 wherein the double-stranded product of step (b) is first purified and then the sense strands of the purified product are digested with an exonuclease to obtain the single-stranded labeled antisense strand in step (c).
9. (currently amended) The method of ~~claim 1~~ claim 22 wherein said single control gene is GAPD.
10. (currently amended) The method of ~~claim 1~~ claim 22 wherein the sizes of the probes range from about 25 to about 60 nucleotides and the targeted segments are between about 100 and 200 base pairs long.
11. (currently amended) The method of ~~claim 1~~ claim 22 wherein two microarrays are used in parallel and the imaging results from both are compared as an initial check on the validity of the hybridizing and imaging steps.
- 12-19. (canceled)

20. (previously presented) A method for detecting any one of multiple chromosomal disorders in a single assay, which method comprises the steps of:

a. making a polymerase chain reaction (PCR) mixture by mixing in a vessel components comprising:

(i) eukaryotic genomic DNA;

(ii) a plurality of different pairs of forward and reverse DNA primer oligonucleotides wherein one primer of each said pair is complementary to a 3' sequence of a targeted segment of a first eukaryotic DNA strand and the other primer is complementary to the 3' sequence of the second strand of the target segment, the length of the segment of eukaryotic DNA being between about 100 and about 250 base pairs, wherein one of the primers of each pair has a color-detectable label attached at the 5' end thereof, and wherein of said plurality of different pairs of primers there are some pairs that are targeted to segments of selected different chromosomes of interest which are indicative of potential chromosomal disorders and one pair that is targeted for a segment of a single control gene; and

(iii) PCR buffers and enzymes necessary to carry out PCR amplification;

b. conducting a PCR for between about 5 and about 60 temperature cycles to create amplified PCR products;

c. purifying said products of step (b) and obtaining single-stranded DNA having the color-detectable labels by digestion of one strand of the amplified double-stranded PCR product,

d. contacting a microarray with products of step (c), which microarray has a plurality of spots that each contain DNA oligonucleotide probes having nucleotide sequences complementary to a nucleotide sequence of one of said strands of said targeted segments;

e. hybridizing said DNA oligonucleotide probes in said microarray and said PCR-amplified label-containing single-stranded products;

f. detecting the presence and relative quantity of the PCR-amplified products hybridized to the microarray by colorimetric imaging of the microarray; and

g. diagnosing whether or not a chromosomal disorder exists with respect to one or more of said different chromosomes of interest by first comparing said imaging of a relevant spot on said microarray for each said chromosome of interest to the imaging of a spot relevant to said single control gene to obtain an I-ratio; then comparing each I-ratio to N-ratios that have been obtained as a result of similar testing of multiple samples of genomic DNA known to be normal, said N-ratios being averages for normal DNA of persons of that gender, of the ratios of intensity of each said segment for each said chromosome of interest to the intensity of said single control gene, wherein said I-ratios are subjected to rule-based algorithms so that each I-ratio is adjusted prior to its use in final diagnosis by using an average C-factor which is obtained after first comparing all of the I-ratios with the respective N-ratios to obtain individual C-factors.

21. (canceled)

22. (previously presented) A method for detecting any one of a plurality of different chromosomal disorders in a single assay, which method comprises the steps of:

a. making a polymerase chain reaction (PCR) mixture by mixing in a vessel components comprising:

(i) eukaryotic genomic DNA;

(ii) different pairs of forward and reverse DNA primer

oligonucleotides designed to amplify each of a plurality of target segments wherein one primer of each said pair is complementary to a 3' sequence of a targeted segment of between about 100 and about 250 base pairs of a first eukaryotic DNA strand and the other primer is complementary to the 3' sequence of the second strand of such target segment, wherein one of the primers of each pair has a detectable label attached at the 5' end thereof, and wherein said different pairs of primers include a plurality of pairs that are targeted to selected segments of different chromosomes which are of interest from the

standpoint of different potential chromosomal disorders and one pair that is targeted for a segment of a single control gene; and

(iii) PCR buffers and enzymes necessary to carry out PCR amplification;

b. conducting a PCR for between about 5 and about 60 temperature cycles to create amplified PCR products;

c. purifying said amplified PCR products and obtaining single-stranded DNA having the labels by digestion of the sense strand of the amplified double-stranded PCR product;

d. contacting a microarray with the resulting antisense products of step (c), which microarray has a plurality of spots that each contain DNA oligonucleotide probes having nucleotide sequences that are complementary to the nucleotide sequences of one of said label-carrying strands of said targeted segments;

e. hybridizing said DNA oligonucleotide probes in said microarray and said PCR-amplified label-containing single-stranded products;

f. detecting the presence and relative quantity of the PCR-amplified products hybridized to the microarray by imaging of the microarray; and

g. diagnosing whether or not a chromosomal disorder exists with respect to one or more of said plurality of chromosomes of interest on which there were targeted segments by:

(i) first comparing said imaging of relevant spots on said microarray for each said chromosome of interest to the imaging of spots relevant to said single control gene to obtain a plurality of I-ratios;

(ii) comparing each of said plurality of I-ratios with the respective N-ratio that is average for normal DNA of persons of that gender to obtain a plurality of C-factors;

(iii) obtaining an average C-factor from said plurality of individual C-factors by averaging all of said individual C-factors between 0.75 and 1.25;

(iv) dividing each said I-ratio by said average C-factor to obtain an adjusted I-ratio (A-ratio) for each; and

(v) then comparing each A-ratio for each of said plurality of chromosomes of interest to said respective N-ratio for that chromosome to determine the difference from normal.